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TI Directed selection of differentiation mutants of Streptomyces noursei using chemostat cultivation
AU Noack, D.
CS Forschungsbereich Biowiss. Med., Dtsch. Akad. Wiss., Jena, DDR-6900, Ger. Dem. Rep.
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FUNDAMENTAL STUDIES OF URINARY TRACT INFECTION WITH SERRATIA INTERACTION BETWEEN BACTERIA OF DIFFERENT GENERA AND SPREADING OF R PLASMID TO SERRATIA.
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CS DEP. UROL., HIROSHIMA UNIV. SCH. MED.
SO MED J HIROSHIMA UNIV, (1986) 34 (4), 453-472.
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FS BA; OLD
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Adaptive reversion of a frameshift mutation in Escherichia coli
AU Cairns, John; Foster, Patricia L.
CS Dep. Cancer Biol., Harvard Sch. Public Health, Boston, MA, 02115, USA
SO Genetics (1991), 128(4), 695-701
CODEN: GENTAE; ISSN: 0016-6731

TI Modification in penicillin-binding proteins during in vivo development of genetic competence of Haemophilus influenzae is associated with a rapid change in the physiological state of cells
AU Dargis, M.; Gourde, P.; Beauchamp, D.; Foiry, B.; Jacques, M.; Malouin, F.
CS Cent. Rech., Cent. Hosp., Ste-Foy, PQ, G1V 4G2, Can.
SO Infect. Immun. (1992), 60(10), 4024-31

I Decreased susceptibilities to teicoplanin and vancomycin among coagulase-negative methicillin-resistant clinical isolates of staphylococci
AU Sieradzki, Krzysztof; Villari, Paolo; Tomasz, Alexander
CS The Rockefeller University, New York, NY, 10021, USA
SO Antimicrobial Agents and Chemotherapy (1998), 42(1), 100-107

Triclosan and antibiotic resistance in Staphylococcus aureus
AU Suller, M. T. E.; Russell, A. D.
CS Pharmaceutical Microbiology, Welsh School of Pharmacy, Cardiff University, Cardiff, CF10 3XF, UK
SO Journal of Antimicrobial Chemotherapy (2000), 46(1), 11-18
CODEN: JACHDX; ISSN: 0305-7453

NO

TI Augmentation of antibiotic resistance in Salmonella typhimurium DT104 following exposure to penicillin derivatives
AU Carlson, S. A.; Ferris, K. E.
CS National Animal Disease Center, Pre-harvest Food Safety and Enteric Disease Research Unit, Agricultural Research Service, USDA, Ames, IA, USA
SO Veterinary Microbiology (2000), 73(1), 25-35

TI Augmentation of antibiotic resistance in Salmonella typhimurium DT104 following exposure to penicillin derivatives

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Directed selection of differentiation mutants of *Streptomyces noursei* using chemostat cultivation

D. Noack

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A nourseothricin-producing *Streptomyces noursei* strain was continuously cultivated in a chemostat equipped with a stirrer for mechanical fractionation of the mycelium. Different cultivation conditions allowed the selection of six types of differentiation mutants after the culture had reached a population genetically stationary state. The mutants showed an altered control pattern of sporulation as well as altered antibiotic biosynthesis and antibiotic resistance. In addition, the stability of the recombinant plasmid pIJ385 in several differentiation type mutants as host strains was tested. The results suggest that there exists a strong correlation between the cultivation conditions employed and the type of differentiation mutants selected.

Genetic studies with Streptomycetes revealed phenotypic alterations pointing to genetic instabilities mainly in the secondary metabolism. This has been demonstrated for example by Schrempf (1982a), Yoshikawa *et al.* (1982) and Ochi *et al.* (1984). Several authors were able to show correlations between the altered phenotype and structural changes in the chromosomal DNA (Hintermann *et al.* 1981, Ono *et al.* 1982, Crameri *et al.* 1983, Fishman and Hershberger 1983, Altenbuchner and Cullum 1984). Transposons have been suggested to be involved in the alterations (Sermonti *et al.* 1983, Nakano *et al.* 1984). For about ten years plasmids are known to possibly take part in the control of secondary metabolism. The observation that plasmids interact with chromosomal DNA (Bibb *et al.* 1981, Schrempf 1982b, Hopwood *et al.* 1984) supports this assumption. Continuous cultivation in a chemostat is the method of choise to quantitative study genetic segregation processes (for review Dykhuizen and Hartl 1983). In our laboratory we adapted the chemostat for a continuous cultivation of mycelialy growing Streptomycetes (Roth and Noack 1982) in order to obtain genetic segregation kinetics concerning the loss of antibiotic forming capacity (Roth *et al.* 1982a, Noack *et al.* 1982). Based on the observation that plasmid DNA is eliminated from *E. coli* host strains in dependence on cultivation conditions present in the chemostat (Noack *et al.* 1981, Wouters and van Andel 1983, Jones and Melling 1984) we investigated the maintenance of the recombinant plasmid pIJ2 in *Streptomyces lividans* in parallel experiments (Roth *et al.* 1985).

In order to study segregation of both chromosomal and extrachromosomal DNA of an antibiotic-producing *Streptomyces* strain, we carried out a number of chemostat cultivations. For these experiments we used the strain *Streptomyces noursei* IMET 3890b NG13 (for reference Friedrich *et al.* 1984) which is the producer of the streptothricin antibiotic nourseothricin. Six types of differentiation mutants were selected after th echemostat culture has reached a stationary state with respect to their genetic composition. These mutants were quantitatively characterized with respect to the control of antibiotic formation and resistance as well as the kinetics of their enrichment within the culture and the elimination of the recombinant plasmid pIJ385.

Materials and methods

Bacterial strain and plasmid: *Streptomyces noursei* JA3890b NG13 was received from the culture collection of this institute (Friedrich *et al.* 1984). Plasmid pIJ385 conferring resistance to neomycin and thiostrepton was provided by D. A. Hopwood (Kieser *et al.* 1982).

Media and growth conditions for chemostat culture: Mineral salts medium for continuous cultivation contained (g per l): KH_2PO_4, 2.72; $Na_2HPO_4 \cdot 12\ H_2O$, 7.16; NaCl, 5.097; Na_2SO_4, 1.065; $MgCl_2 \cdot 6\ H_2O$, 0.04; $FeCl_3$, 0.005; $MnCl_2$, 0.004; NH_4Cl, 0.4 (for ammonium limitation 0.08); glucose, 2.5 (for glucose limitation 0.5). pH was adjusted at 7.0. Glucose was separately autoclaved and then added to the medium.

The chemostat for continuous cultivation of *Streptomyces* strains has been described previously (Roth and Noack 1982).

For simultaneous limitation with both ammonium chloride and maltose the culture medium contained ammonium chloride 0.08 g/l and maltose 0.5 g/l instead of glucose. Experiments were started by inoculating a 15 ml batch preculture of the respective strain into the chemostat vessel. To obtain the preculture a small amount of surface mycelium was withdrawn from a 7 days old surface culture on M79 medium and incubated for 48 hrs on a rotatory shaker at 28 °C.

Determination of nourseothricin formation: A loop full of surface mycelium of the respective strain was inoculated into 15 ml of mineral salts medium supplemented in excess with ammonium chloride and glucose and cultivated for 2 days on a rotatory shaker at 28 °C. Four droplets of about 2 µl of the culture suspension were then placed onto the surface of antibiotic test agar in a distance of about 20 mm. After 2 days of incubation at 28 °C these plates were overlayed with 5 ml of medium Al53 containing 0.05 ml of a spore suspension of a *Bacillus subtilis* tester strain. After 16 hrs of incubation at 37 °C the diameter of inhibition zones were measured surrounding the colonies that arose from the droplets.

Content of plating media (g per l): AL53: Saccharose, 3; dextrin, 15; urea, 0.1; bacto peptone, 5; yeast extract, 1; NaCl, 0.5; KH_2PO_4, 0.5; $FeSO_4 \cdot 7\ H_2O$, 0.01; agar, 0.15.

M79: Glucose, 10; bacto peptone, 10; casamino acids, 1; yeast extract, 2; NaCl, 6; agar, 0.2.

MM: Mineral salts medium as for chemostat culture with NH_4Cl in excess and without glucose; agar, 0.2.

MY: MM supplemented with 0.2% yeast extract.

MYO: MY supplemented with KH_2PO_4 to a final concentration of 0.06 molar.

MG: MM supplemented with 1% glucose.

MGO: MG supplemented with KH_2PO_4 to a final concentration of 0.06 molar.

WAY: Agar, 0.2; supplemented with 0.2% yeast extract.

WAYG: WAY supplemented with 1% glucose.

Determination of antibiotic resistance: Liquid cultures as for determination of nourseothricin formation were spread onto M79. After 4 hrs of incubation at 28 °C wells of 8 mm diameter were punched and filled with 0.05 ml of antibiotic solutions. The antibiotics neomycin (Nm), thiostrepton (Ts) and nourseothricin (Nt) were used at the following concentrations 1000 µg/ml, 3000 µg/ml and 10000 µg/ml. The diameter of inhibition zones surrounding the wells was measured after additional incubation for 3 days at 28 °C.

Results

Continuous cultivation

The nourseothricin-producing *Streptomyces noursei* 3890b NG13 was continuously cultivated in a chemostat endowed with a high-speed stirrer to fractionate the mycelium to pieces of an overall mycelial length of about 100–200 µm.

During prolonged cultivation the genotypic composition of the mycelial population changed due to the enrichment of preexisting and/or spontaneously appearing mutants having a growth advantage under the limitation conditions employed in the chemostat. The population tended to a genotypic steady state which is proposed to be reached when at least 99.9% of the colony-forming units withdrawn from the chemostat culture exhibited the same phenotype as characterized by growth pattern, control of antibiotic formation and antibiotic resistance.

In all cases studied this population genetic steady state was established after 100 generations, i. e. 100 doublings of biomass which can be calculated by $N = \frac{T \cdot D}{\ln 2}$, where N is the number of generations, T the cultivation time and D the dilution rate.

Colony-forming units withdrawn from the chemostat after at least 100 generations were tested for the properties mentioned above. Previous results (Roth and Noack 1982, Roth *et al.* 1982a, Noack *et al.* 1982) together with those presented here allow to propose a strong correlation between the cultivation conditions realized in the chemostat and the population genetic steady state established after at least 100 generations.

Directed selection of differentiation mutants

Fig. 1 demonstrates the genealogy of differentiation mutants selected after chemostat cultivations. Steps carried out under limitation conditions are marked by solid arrows. The dotted arrows represent transformation with plasmid pIJ385 resulting in Nm^R and Ts^R clones marked by letter "T".

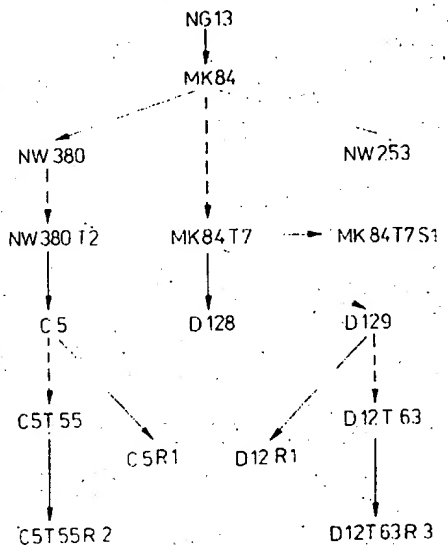

Fig. 1
Relation scheme of differentiation mutants selected from chemostat cultures. Solid arrows represent selection procedures based on different cultivation conditions. Dotted arrows stand for transformation with plasmid pIJ385 DNA.

The properties of differentiation mutants are listed in Table 1. The parameters of segragation kinetics resulting in the enrichment of the mutants are listed in Table 2. The parameters characterizing the kinetics of the segregation of plasmid pIJ385 are listed in Table 3.

In the first step the strain NG13 was cultivated under ammonium chloride limitation at $T = 28$ °C and $D = 0.15$ h^{-1} in order to select differentiation mutants adapted to chemostat cultivation. After a cultivation time corresponding to 150 generations mutants with an altered phenotype appeared which failed to form aerial mycelium and spores. Mutants showing this altered phenotype did not revert after several passa-

ges both in liquid and on solid medium, therefore, it is designated as to be an altered genotype. The properties of one representive clone, MK84, are listed in Table 1. The amounts of antibiotic produced were calculated from inhibition zones appearing on antibiotic test agar. They are listed as relative values with the highest one taken as 100%. The same principle was applied to calculate the relative level of antibiotic resistance. All the values listed in Table 1 are means of at least 6 independent experimental data and include mean errors of $\pm 2\%$.

The differentiation mutant MK84 produced more antibiotic than the original strain NG13 on the test medium MG and MY. It was, however, drastically depressed on M79 agar. The productivity is some what less depressed on the media WAY and WAYG which were thought to replace M79. The sensitivity of antibiotic production to high

Table 1
Properties of differentiation mutants selected from chemostat cultures. The values listed are relative ones as explained in the Materials and methods section. The column "spo" indicates the ability to form aerial mycelium and spores (+) or the respective failure (−). The letters A to G stand for differentiation types which can be distinguished by their control pattern for sporulation, nourseothricin biosynthesis and nourseothricin resistance. The mutants containing plasmids are indicated by primed (') letters.

Strain	MG	MGO	MY	MYO	M79	WAY	WAYG	Nm^R	Nt^R	Spo	Diff. Type
NG13	70	55	90	80	65	55	15	30	100	+	A
MK84	85	35	100	70	5	35	5	30	100	−	B
MK84T7	55	20	85	40	4	25	5	100	100		B'
NW253	10	5	15	10	10	8	5	30	65	−	C
NW380	55	45	80	65	10	8	5	30	100	−	D
NW380T2	45	35	65	55	8	8	5	100	100	−	D'
MK84T7S1	75	35	100	65	12	40	10	30	100	−	E
C5	0	0	0	0	15	0	10	30	20	−	F
D128	0	0	0	0	15	0	10	30	20	−	F
D129	0	0	0	0	15	0	10	30	20	−	F
C5T55	0	0	0	0	10	0	5	70	25	−	F'
D12T63	0	0	0	0	10	0	5	70	25	−	F'
C5R1	85	35	100	75	15	40	20	30	100	−	G
D12R1	85	35	100	75	15	40	20	30	100	−	G
D5T55R2	80	30	95	70	10	35	15	70	100	−	G'
D12T63R3	80	30	95	70	10	35	15	70	100	−	G'

phosphate concentrations was markedly enhanced on medium MGO and MYO. The level of resistance to Nm and Nt did not change. Separate experiments (KRÜGEL *et al.* 1985) showed that the differentiation mutant MK84 had aquired several new properties turning it into an excellent recipient for recombinant DNA experiments.

The transition from the population originally inoculated into the chemostat and the derivative population established after prolonged cultivation may be described by plotting the logarithm of the ratio of colony-forming units of the former genotype against the number of generations calculated on the basis of dilution rate and cultivation time. This segregation kinetics can be described by two parameters: the segregation rate δ of the original genotype into the segregated one and the selection pressure σ referring to the growth advantage of the segregated genotype over the original one (NOACK *et al.* 1984). The respective values in relation to the generation time τ are shown in the first lane of Table 2.

The next selection procedure was carried out with glucose limitation in order to obtain differentiation mutants defective in antibiotic synthesis. However, only a drastic decrease of antibiotic productivity was observed with the representative clone

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YG	Nm^R	Nt^R	Spo	Diff. Type
	30	100	+	A
	30	100	+	B
	100	100	+	B'
	30	65	—	C
	30	100	—	D
	100	100	—	D'
	30	100	+	E
	30	20	—	F
	30	20	—	F
	30	20	—	F
	70	25	—	F'
	70	25	—	F'
	30	100	+	G
	30	100	+	G
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Table 2
Characteristic parameters of segregation kinetics concerning chromosomally encoded determinants of differentiated functions.
The segregation kinetics of chromosomal markers involved in the control of sporulation or nourseothricin formation or nourseothricin resistance were obtained by plotting in a semilogarithmic scale the ratio of colony-forming units of the parental type against the number of generations i.e. the number of doublings of biomass. From the shape of this kinetics the segregation rate δ and the selection pressure σ were calculated according to the mathematical method described by NOACK *et al.* (1984). The first four columns show the growth parameters realized in the chemostat. The fifth and the last column contain the parental strains and the differentiation mutants. The cases where no segregation could be observed are marked with (0) and (—) in the respective column.

Limiting substrate	Substrate in excess	Dilution rate [h^{-1}]	Temperature	Strain inoculated	Segregation rate	Selection pressure	Selected strains
NH_4Cl	Glucose	0.15	28 C	NG13	$3.2 \cdot 10^{-2}$	$9.6 \cdot 10^{-2}$	MK84
Glucose	NH_4Cl	0.15	28 C	MK84	$2.5 \cdot 10^{-3}$	$2.5 \cdot 10^{-1}$	NW253
NH_4Cl	Maltose; PO_4	0.15	37 C	MK84	not determ.	not determ.	NW380
Glucose	NH_4Cl	0.12	28 C	MK84T7	$5.6 \cdot 10^{-4}$	$7.3 \cdot 10^{-2}$	D128; D129
Glucose	NH_4Cl	0.12	28 C	NW380T2	$2.0 \cdot 10^{-4}$	$5.6 \cdot 10^{-2}$	C5
NH_4Cl	Glucose	0.12	28 C	MK84T7	0	—	
NH_4Cl	Glucose	0.12	28 C	NW380T2	0	—	
Malt. + NH_4Cl	Phosphate	0.12	37 C	MK84T7	0	—	
Malt. + NH_4Cl	Phosphate	0.12	37 C	NW380T2	0	—	

NW253 onto all media (lane 4 of Table 1). The level of Nt-resistance was markedly reduced. The spo⁻ phenotype has not been changed.

The segregation kinetic data (lane 2 of Table 2) show that strain NW253 appeared with a rate δ per generation τ one order of magnitude lower than the derivative MK84. In contrast the selection pressure σ remained at about the same order.

The strain MK84 was subjected to a selection program with maltose as carbon and energy source in order to get derivatives adapted to the use of oligosaccharides instead of glucose (Fig. 1 and lane 3 of Table 2). Simultaneous limitation with ammonium chloride was applied to avoid the appearance of strains drastically depressed in nourseothricin biosynthesis. Enhancement of the phosphate concentration up to 0.06 molar (instead of 0.02 molar in the basic medium) was expected to result in the selection of strains with a stabilized phosphate resistance of the nourseothricin biosynthesis. One representative strain, NW380, was isolated after 150 generations of cultivation. It exhibited a slightly increased resistance to higher phosphate concentrations as compared to strain MK84. However, the absolute level of antibiotic productivity remained below that of MK84. The parameters of segregation kinetics could not be determined because of the small differences in antibiotic productivities between MK84 and NW380.

Both selectants MK84 and NW380 were transformed with plasmid pIJ385 (dotted arrows in Fig. 1). The transformants MK84T7 and NW380T2 were resistant to neomycin and thiostrepton and contained a plasmid which showed no differences to pIJ385. These strains were subjected to chemostat cultivation with glucose limitation in order to test the stability of both the plasmid inheritance and the antibiotic productivity.

Table 3
Characteristic parameters of segregation kinetics concerning plasmid-encoded neomycin resistance. The segregation kinetics of plasmid pIJ385 DNA were obtained by plotting in a semilogarithmic scale the ratio of Nm-resistant colony-forming units against the number of generations. The segregation parameters δ and σ were calculated as described above.

Limiting substrate	Substrate in excess	Dilution rate [h^{-1}]	Temperature	Strain inoculated	Segregation rate	Selection pressure
Glucose	NH_4Cl	0.12	28 C	MK84T7	$2.3 \cdot 10^{-4}$	$7.4 \cdot 10^{-2}$
Glucose	NH_4Cl	0.12	28 C	NW380T2	$2.5 \cdot 10^{-2}$	$4.5 \cdot 10^{-2}$
NH_4Cl	Glucose	0.12	28 C	MK84T7	0	—
NH_4Cl	Glucose	0.12	28 C	NW380T2	0	—
Malt. NH_4Cl	Phosphate	0.12	37 C	MK84T7	$5.6 \cdot 10^{-3}$	$1.7 \cdot 10^{-2}$
Malt. NH_4Cl	Phosphate	0.12	37 C	NW380T2	$8.8 \cdot 10^{-3}$	$1.3 \cdot 10^{-2}$

As shown in Table 3 (lane 1 and 2) the neomycin resistance was lost from strain MK84T7 with a segregation rate δ three orders of magnitude smaller than that of strain NW380. All neomycin sensitive selectants tested had lost the plasmid pIJ385. The selection pressure σ remained at the same level.

The plasmid-free strain MK84T7S1 included in Fig. 1 and Table 1 resulted from spontaneous loss of pIJ385 during batch cultivation in complete medium.

The test for antibiotic productivity during glucose-limited cultivation of both MK84T7 and NW380T2 revealed an enrichment of clones totally represented in nourseothricin biosynthesis on most of the media used. The antibiotic formation of such strains, e. g. C5, D128 and D129 seemed to be controlled in a completely different way than in the parental strains since they produced only trace amounts of nourseothricin on M79 and WAYG. In addition, the level of nourseothricin resistance was drastically decreased indicating a qualitative change in the resistance mechanism. The segregation rates for both strains were nearly identical and extremely low being only about 10^{-4} per generation.

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ain culated	Segregation rate	Selection pressure
84T7	$2.3 \cdot 10^{-4}$	$7.4 \cdot 10^{-2}$
V380T2	$2.5 \cdot 10^{-4}$	$4.5 \cdot 10^{-2}$
84T7	0	—
V380T2	0	—
84T7	$5.6 \cdot 10^{-3}$	$1.7 \cdot 10^{-2}$
V380T2	$8.8 \cdot 10^{-3}$	$1.3 \cdot 10^{-2}$

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Separate experiments (Haupt *et al.* 1986) showed that these derivatives lost the ability to enzymatically acetylate the nourseothricin molecules. These properties did not change after transformation with plasmid pIJ385 which resulted in strains C5T55 and D12T63, respectively.

It has to be emphasized that in every case plasmid pIJ385 was lost after or at least simultaneously with the determinant for the control of nourseothricin biosynthesis and resistance. However, no plasmid-containing strain with the phenotype of C5, D128 and D129 was isolated because the selection of differentiation mutants took place only after 150 generations when the ratio of plasmid-containing clones have been dropped below 10^{-2}.

Retransformation with plasmid pIJ385 (dotted arrows in Fig. 1) did not influence the altered phenotype (see the data for C5T55 and D12T63 in Table 1). However, the expression of Nm-resistance was markedly reduced.

In order to test the stability of this drastically altered phenotype, the plasmid-free as well as the plasmid-bearing differentiation mutants were batch cultivated in the complex liquid medium MY. After repeated transfer into fresh medium for about 100 generations few revertants appeared with drastically enhanced Nt-resistance. These revertants simultaneously retained their Nt-forming proficiency to form Nt at a level which was at least comparable to that of the parental strains MK84 and NW380. Four of these revertants C5R1, D12R1, C5T55R2 and D12T63R3 are listed in Table 1.

In previous experiments (Roth and Noack 1982, Roth *et al.* 1982a) with other *Streptomyces* species no segregation of chromosomal determinants for the control of antibiotic synthesis were observed when the strains were continuously cultivated under ammonium limitation at low dilution rates and low temperature. This observation holds also for the strains MK84, MK84T7 and NW380T2 of this species irrespective whether the growth was limited by ammonium chlorid alone or in addition by maltose (last lanes of Table 2). The plasmid was stably inherited during limitation by ammonium chloride alone (lane 3 and 4 of Table 3). However, the additional limitation by maltose as carbon and energy source resulted in the segregation of plasmids from either strain MK84T7 or NW380T2 with nearly the same rate δ.

It should be emphasized that the values of selection pressure calculated from all segregation kinetics obtained did not differ markedly.

Discussion

The nourseothricin-producing strain *Streptomyces noursei* NG13 was continuously cultivated in a chemostat under different limitation conditions. After a least 100 generations corresponding to 100 doublings of biomass a stationary state of the genetic composition of the population was established as reflected by more than 99.9% of cells with an altered phenotype. Six types of differentiation mutants designated B to G (Table 1) were isolated and distinguished from each other by their control characteristic of nourseothricin biosynthesis and antibiotic resistance. The phenotype of these differentiation mutants was stable over at least 6 subcultivations in liquid and/or on solid media in the absence of any selection pressure. The type of differentiation mutant obtained was strongly depended on the selection pressure realized in the chemostat. It was for example expected that the mutant MK84 exhibited a spo$^-$ phenotype and retained its ability to form nourseothricin. On the contrary, the markable alteration in the control pattern of nourseothricin biosynthesis on the media M79, WAY and WAYG was not predictable. From this result and the properties of the other differen-

tiation mutants it can be postulated that a directed selection and enrichment of desired differentiation mutants can be achieved but that these mutants may exhibit several additional properties which are not expected and predicted. These uncertainties result from our limited knowledge of the genetical and physiological reaction of the strain under study on the selection pressure applied in the chemostat.

This principle is also illustrated by the differentiation types C and F. Both of them were selected after glucose limitation. Type C retained a low nourseothricin-forming capacity on all media tested. In contrast the type F strains showed only a very low activity onto media M79 and WAYG. These differences in the segregation behaviour may be due to plasmid pIJ385 originally present in the parental strains MK84T7 and NW380T2. This suggestion was supported by the fact that plasmid pIJ385 was eliminated from MK84 and NW380 with quite different segregation rates (lane 1 and 2 of Table 3) although the same limitation condition was present in the chemostat. In addition, pIJ385 was eliminated from MK84 and NW380 with nearly identical but intermediate rate when cultivated under simultaneous limitation with maltose and ammonium chloride. Taken together, these facts demonstrate that the kinetics of elimination plasmids from *Streptomyces* strains depends on both the selection pressure employed within the chemostat and the genotype of the host strain.

As it could be predicted from earlier experiments, the limitation with ammonium chloride did not result in the segregation of both chromosomal and extrachromosomal determinants for the control of antibiotic synthesis and antibiotic resistance.

Differentiation mutants of types F and F′ were obviously not perfectly stable but segregated clones of types G and G′, respectively, which both had a somewhat higher nourseothricin-forming capacity and nourseothricin resistance than the parental types B′ and D′. This observation may allow to design a strategy for the improvement of industrial strains used to produce antibiotics. The appearance of G and G′ revertants was obviously restricted to spontaneous processes in batch cultures on complex media because in chemostat cultures the antibiotic producing clones have a growth disadvantage with respect to those not able to form antibiotic.

In conclusion it can be postulated that desired differentiation mutants of streptomycetes may be selected with the aid of a chemostat. These mutants may exhibit additional properties which so far can not exactly be predicted and which seem to depend strongly on both the interrelation between the limitation condition realized in the chemostat and the genotype of the strain under study. Further improvement of our knowledge will allow a better predication of desired differentiation mutants to be selected in the chemostat.

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m types C and F. Both of them
d a low nourseothricin-forming
strains showed only a very low
es in the segregation behaviour
e parental strains MK84T7 and
that plasmid pIJ385 was elimi-
gregation rates (lane 1 and 2 of
s present in the chemostat. In
80 with nearly identical but in-
s limitation with maltose and
onstrate that the kinetics of eli-
on both the selection pressure
e host strain.
the limitation with ammonium
nosomal and extrachromosomal
l antibiotic resistance.
iously not perfectly stable but
h both had a somewhat higher
resistance than the parental
a strategy for the improvement
appearance of G and G′ rever-
s in batch cultures on complex
oducing clones have a growth
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